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The relationship between plasma lipid peroxidation products and primary graft dysfunction after lung transplantation is modified by donor smoking and reperfusion hyperoxia

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Abstract

Purpose—Donor smoking history and higher FiO_2 at reperfusion are associated with primary graft dysfunction (PGD) after lung transplantation. We hypothesized that oxidative injury biomarkers would be elevated in PGD, with higher levels associated with donor exposure to cigarette smoke and recipient hyperoxia at reperfusion.

Methods—We performed a nested case control study of 72 lung transplant recipients from the Lung Transplant Outcomes Group cohort. F_2-isoprostanes and isofurans were measured by mass spectroscopy in plasma collected after transplantation. Cases were defined in two ways: grade 3 PGD present at day 2 or day 3 after reperfusion (severe PGD), or any grade 3 PGD (any PGD).

Results—There were 31 severe PGD cases with 41 controls and 35 any PGD cases with 37 controls. Plasma F_2-isoprostane levels were higher in severe PGD compared to controls (28.6 pg/ml vs. 19.8 pg/ml, p=0.03). Plasma F_2-isoprostane levels were higher in severe PGD compared to controls (29.6 pg/ml vs. 19.0 pg/ml, p=0.03) among patients reperfused with FiO_2>40%. Among recipients of lungs from donors with smoke exposure, plasma F_2-isoprostane (38.2 pg/ml vs. 22.5 pg/ml, p=0.046) and isofuran (66.9 pg/ml vs. 34.6 pg/ml, p=0.046) levels were higher in severe PGD compared with controls.

Conclusions—Plasma levels of lipid peroxidation products are higher in patients with severe PGD, in recipients of lungs from donors with smoke exposure, and recipients exposed to higher FiO_2 at reperfusion. Oxidative injury is an important mechanism of PGD and may be magnified by donor exposure to cigarette smoke and hyperoxia at reperfusion.

Introduction

Primary graft dysfunction (PGD) is a form of acute lung injury predominantly resulting from severe ischemia reperfusion injury (IRI) in the allograft in the setting of lung transplantation[1-3]. We have previously reported that receipt of a lung from a donor with smoking exposure history as well as higher fraction of inspired oxygen (FiO_2) at the time of organ reperfusion are independent risk factors for the development of PGD[4]. The mechanisms leading to these associations are unclear.

Exposure to both cigarette smoke and hyperoxia can induce oxidative injury in the lung[5,6]. Although the role of reactive oxygen species (ROS) in signaling and lung homeostasis is complex, during lung transplantation there may be an excess of ROS production, leading to lipid peroxidation[7]. F_2-isoprostanes are a group of prostaglandin-like compounds formed via nonenzymatic free radical-induced peroxidation of arachidonic acid that serve as robust markers of oxidative lipid peroxidation[8]. Some of these compounds have other physiologic effects in the lung including vasoconstriction of the pulmonary vasculature[9]. F_2-isoprostane

levels are higher in smokers compared to non-smokers and decrease in response to smoking cessation and antioxidant therapy[10,11]. In animal models of ischemia and reperfusion of the heart and liver, F_2-isoprostanes are released in response to reperfusion[12-14]. In rabbit lungs, hyperoxia and anoxia induced release of F_2-isoprostanes[15]. Plasma F_2-isoprostane levels have also been associated with organ dysfunction in patients with severe sepsis[16].

Isofurans are also formed as a result of lipid peroxidation of arachidonic acid, but differ from F_2-isoprostanes by the presence of a tetrahydrofuran ring[17]. The formation of isofurans and F_2-isoprostanes share an intermediate step that is dependent on oxygen concentration. In the presence of high oxygen tension, isofuran formation is favored[18,19]. Isofurans may thus play a role in hyperoxia-induced oxidant injury[18].

Because oxidant injury and lipid peroxidation may be important mechanistic pathways in PGD, we hypothesized that higher systemic F_2-isoprostane and isofuran concentrations would be associated with PGD. Furthermore, based on the association of F_2-isoprostanes and isofurans with hyperoxia and cigarette smoke exposure, we hypothesized that the association of F_2-isoprostanes and isofurans with PGD would be impacted by donor smoking history and higher FiO_2 at reperfusion.

Materials and Methods

Subject selection and study design

A nested case control study was chosen for efficiency based on the cost of performing the bioassays of lipid peroxidation. Subjects were selected from lung transplant recipients enrolled in the prospective multicenter Lung Transplant Outcomes Group (LTOG) cohort between 2002 and 2012[4,20]. PGD cases were defined utilizing the International Society for Heart and Lung Transplantation guidelines in two ways: 1) grade 3 PGD present at day 2 or day 3 after reperfusion (severe PGD); 2) any grade 3 PGD in the first 72 hours after allograft reperfusion (any PGD) [2,4,21-24]. The severe PGD definition is thought to represent a more severe phenotype, associated with worse mortality, and has been utilized in previous studies[4]. Cases and controls were selected to ensure inclusion of subjects who had received allografts from both donors with and without a history of cigarette smoke exposure as well as a broad range of fraction of inspired oxygen (FiO_2) at the time of allograft reperfusion. Donor smoke exposure was defined as any reported current or former history of donor cigarette use, collected prospectively from multiple sources at the time of transplant, including UNet data. FiO_2 at reperfusion was defined as the value recorded in the anesthesia clinical record at the time of allograft reperfusion[4]. The number of controls was limited by the small number of non-PGD patients receiving an allograft from a previously non-smoking donor in the overall LTOG cohort. Subjects had blood samples collected 6 hours after allograft reperfusion in citrated tubes; samples were processed within 30 minutes and then stored at −80°C as previously described[25-28]. Clinical information regarding donor, recipient, and perioperative characteristics and events were prospectively recorded on standardized case report forms[4,20]. The institutional review boards at all participating centers approved the study.

Plasma F_2-isoprostane and isofuran measurement

Plasma F_2-isoprostane and isofuran levels were measured in duplicate by stable isotope dilution negative ion chemical ionization gas chromatography mass spectrometry[29]. There was no association between sample storage time and plasma concentration measurements. All laboratory personnel were blinded to the PGD status of the recipients.

Statistical Analysis

Donor, recipient, and peri-operative demographics were compared utilizing Student's t-test or Chi-squared analysis as appropriate. Plasma F_2-isoprostane and isofuran concentrations were log transformed for statistical analyses in order to account for non-normal distributions. Differences in mean transformed concentrations were evaluated using two-sided Student's t-test. Reported plasma concentrations correspond to the mean transformed concentrations. There was >90% power to detect a 1 standard deviation difference in transformed plasma concentration. We utilized a priori subgroup analysis in order to evaluate the impact of reperfusion FiO_2 and donor smoke exposure on the association of plasma F_2-isoprostanes and isofurans with PGD. All statistical analysis was performed using STATA 13.1 software (STATA Corp., College Station, TX); GraphPad Prism 6 (GraphPad Software, La Jolla, CA) was used for generating graphs. A p-value <0.05 was used to determine significance.

Results

Severe PGD case definition

There were 31 severe PGD cases and 41 non-PGD controls. Table 1 describes the donor, recipient, and perioperative characteristics. Recipients with severe PGD had significantly higher mean body mass index (BMI) compared to controls (27.4 vs. 24.0, p<0.001), and other risk factor variables were similarly distributed as in prior studies[4]. Plasma F_2-isoprostane levels were significantly higher in patients with severe PGD compared to those without PGD (28.6 pg/ml vs. 19.8 pg/ml, p=0.03). There was no significant difference in plasma isofuran levels according to PGD status (39.7 pg/ml vs. 31.9 pg/ml, p=0.2) (Figure 1).

The impact of reperfusion FiO_2 and donor smoke exposure history on the association between lipid peroxidation products and severe PGD was assessed using subgroup analyses. In patients reperfused with FiO_2>40%, plasma F_2-isoprostane levels were significantly higher in patients with severe PGD compared to those without PGD (29.6 pg/ml vs. 19.0 pg/ml, p=0.03). In contrast, there was no association between plasma F_2-isoprostane levels and severe PGD in subjects reperfused at FiO_2≤40% (24.9 pg/ml vs. 21.5 pg/ml, p=0.6). There was no detected association of plasma isofuran levels with severe PGD in subjects reperfused with either FiO_2>40% (39.3 pg/ml vs. 34.0 pg/ml, p=0.5) or FiO_2≤40% (41.2 pg/ml vs. 28.2 pg/ml, p=0.3) (Figure 2).

Among patients receiving a lung from a donor with cigarette smoke exposure, plasma F_2-isoprostane levels were significantly higher in patients with severe PGD compared to controls (38.2 pg/ml vs. 22.5 pg/ml, p=0.046). There was no association between plasma F_2-

isoprostane levels and severe PGD among patients receiving a lung from a non-smoking donor (23.2 pg/ml vs. 18.1 pg/ml, p=0.3). In subjects receiving a lung from a previously smoking donor, isofuran levels were significantly higher in patients with severe PGD compared to controls (66.9 pg/ml vs. 34.6 pg/ml, p=0.046). In subjects receiving a lung from a non-smoking donor, there was no difference in isofuran levels between patients with and without severe PGD (27.2 pg/ml vs. 30.2 pg/ml, p=0.5) (Figure 3).

Alternate PGD case definition

The demographics of the patients utilizing any PGD as the case definition are presented in Supplemental Table 1. There was an association between plasma F_2-isoprostanes with any PGD (28.8 vs. 19.1, p=0.01) but no association between isofurans (40.1 pg/ml vs. 31.1 pg/ml, p=0.1) and any PGD (Supplemental Figure 1). Among patients reperfused with FiO_2>40%, F_2-isoprostane levels were significantly higher in patients with any PGD compared to control subjects (30.2 pg/ml vs. 18.3 pg/ml, p=0.02). In patients reperfused at lower FiO_2, there was no difference in F_2-isoprostane levels (24.7 pg/ml vs. 21.0 pg/ml, p=0.6). There was no association between isofuran levels and any PGD when evaluating subgroups defined by reperfusion FiO_2. In contrast to the analysis of severe PGD, there was no association between F_2-isoprostane or isofuran levels and any PGD when evaluating subgroups defined by previous donor smoking status.

Discussion

We found that post-reperfusion plasma levels of lipid peroxidation products are associated with severe PGD after lung transplantation. Furthermore, the association between plasma F_2-isoprostanes and PGD differed according to donor smoking history and reperfusion FiO_2. These findings provide possible evidence that previously identified associations between receipt of a lung from a donor with smoke exposure or higher reperfusion FiO_2 and development of PGD involve potentiation of oxidant injury by these pre- and peri-operative exposures[4].

Oxidative injury may be an important mechanism of acute lung injury in PGD that may be potentiated by donor exposure to cigarette smoke. There is a well-documented association between lipid peroxidation products and cigarette exposure[11,30]. For example, thiobarbituric acid reactive substances (TBARS), biomarkers of lipid peroxidation, are doubled in the bronchoalveolar lavage (BAL) fluid of chronic smokers and increased 6-fold in acute smokers compared to non-smoking controls[31]. Thus, the allograft procured from a donor with smoke exposure may be inherently prone to injury due to the accumulation of lipid peroxidation products, and less likely to be able to recover from IRI in the setting of oxidative damage. In support of this, donor smoking history is strongly associated with risk of PGD in both risk factor as well as predictive models[4,32]. Furthermore, donor smoking is associated with increased pulmonary edema, impaired gas exchange, and higher BAL levels of inflammatory chemokines in explanted lungs not used for transplantation[33]. The current finding of association of PGD with high levels of lipid peroxidation products secondary to donor smoke exposure further supports the importance of oxidative injury pathways in PGD. However, given that our results cannot rule out that lipid peroxidation is an epiphenomenon

rather than the underlying cause of lung injury, model systems such as the ex vivo human lung will need to be employed to provide further evidence of causality. Our study provides proof-of-concept to justify such studies.

Higher FiO_2 at reperfusion was also associated with increased lipid peroxidation. Hyperoxia is associated with increased reactive oxygen species production and neutrophil recruitment[34]. In prior studies, both isofuran and isoprostane formation increase with hyperoxia. Isofuran formation increases with increasing oxygen tension in rat lung models and in vitro using arachodonic acid oxidation models[17]. Increasing FiO_2 from 21% to 28% for one hour in healthy human subjects and subjects with COPD resulted in a significant increase in exhaled breath condensate levels of isoprostanes in both groups[35]. Mice exposed to hyperoxia have increased production of pulmonary F_2-isoprostanes and isofurans compared to mice maintained at room air[36]. We previously demonstrated that compared to a reperfusion FiO_2 less than 0.40, reperfusion $FiO_2 \geq 0.40$ was associated with a 6% absolute risk increase for the development of PGD[4]. While the increased risk of PGD with higher reperfusion FiO_2 may simply be a marker of poor allograft function at the time of reperfusion and a need for higher FiO_2 in order to maintain reasonable PaO_2 levels, we have previously demonstrated that there is wide variability of reperfusion FiO_2 across transplant centers and that centers with the lowest PGD incidence reported the lowest mean reperfusion FiO_2[4]. Increased lipid peroxidation in the setting of higher FiO_2 may provide an explanation for the association of hyperoxia at reperfusion with PGD. When combined with previous animal and human studies, the suggestion of differing relationships between lipid peroxidation products and PGD based on reperfusion FiO_2 highlights the need to focus on reducing reperfusion FiO_2 as a possible preventative therapy for patients undergoing lung transplantation.

Lipid peroxidation pathways may be an attractive therapeutic target for decreasing PGD risk. Dietary flaxseed contains lignans, specifically secoisolariciresinol diglucoside, that have been shown to scavenge hydroxyl radicals and inhibit lipid peroxidation[37]. Pre-treatment of mice with dietary flaxseed containing lignans decreased lung tissue production of F_2-isoprostane after IRI in a hilar crossclamp model[38]. Urinary F_2-isoprostane levels decreased in patients with cystic fibrosis after ingestion of dietary flaxseed compared to baseline[39]. Increases in bronchoalveolar lavage isoprostane levels after lung ischemia-reperfusion were attenuated in mice pre-treated with 14-days of azithromycin, suggesting that azithromycin may have an anti-oxidant effect[40]. Maternal tobacco smoke exposure significantly decreases tissue glutathione levels in the lungs of fetal rats. Maternal administration of the anti-oxidant n-acetylcysteine protected rat fetal lungs from oxidative damage and prevented the smoke exposure-related decline in glutathione levels[41]. In a feline cigarette exposure model, tiotropium administration was associated with reduction in serum, lung, and tracheal lipid peroxides and abrogated reductions in antioxidant levels[42]. Tiotropium may increase anti-inflammatory signaling through modification of histone deacetylases (HDAC) and modulation of nitric oxide production[43]. Similarly, treatment with valproic acid, an HDAC inhibitor, during hypoxia resulted in decreased levels of lipid peroxidation biomarkers and enhanced anti-oxidant enzymatic function in newborn rats[44]. Ex vivo lung perfusion technology allows for evaluating therapies in the allograft prior to organ implantation[45,46]. Therefore, lipid peroxidation product levels may be useful biomarkers of therapeutic

efficacy for preliminary trials of dietary flaxseed or lignans, azithromycin, n-acteylcysteine, valproic acid, and tiotropium, therapies with known safety profiles in human studies.

This study has some limitations. The overall sample size is small because measurements of plasma F_2-isoprostanes and isofurans are costly and labor intensive. In order to maximize the power of our study design using small numbers of subjects, we enriched the sample for patients receiving an organ from a previous smoker as well as a wide range of reperfusion FiO_2. The small sample size limits our ability to perform formal tests for interaction, e.g. using multiplicative terms in logistic regression models. Despite the small size and lack of power, a priori sub-group analyses suggest potential effect modification by both donor smoke exposure and reperfusion FiO_2. Given the post-transplant collection of plasma, we are also unable to make definitive conclusions regarding the causal relationship between lipid peroxidation products and PGD. Future studies evaluating longitudinal changes from recipient pre-transplant levels of these products as well as the impact of donor plasma and BAL levels of lipid peroxidation products prior to allograft procurement combined with model systems will be needed to further define the relationship between these products of oxidant stress and PGD.

In summary, plasma levels of lipid peroxidation products are significantly higher in patients with PGD after lung transplantation compared to those who do not develop PGD and there are significant differences within groups defined by donor smoking history and reperfusion FiO_2. This study further highlights the importance of oxidative injury in the development of lung injury after lung transplantation. Future studies should be aimed at evaluating the causal relationship of lipid peroxidation with PGD after lung transplantation and therapies targeting lipid peroxidation pathways and products in order to prevent the development of PGD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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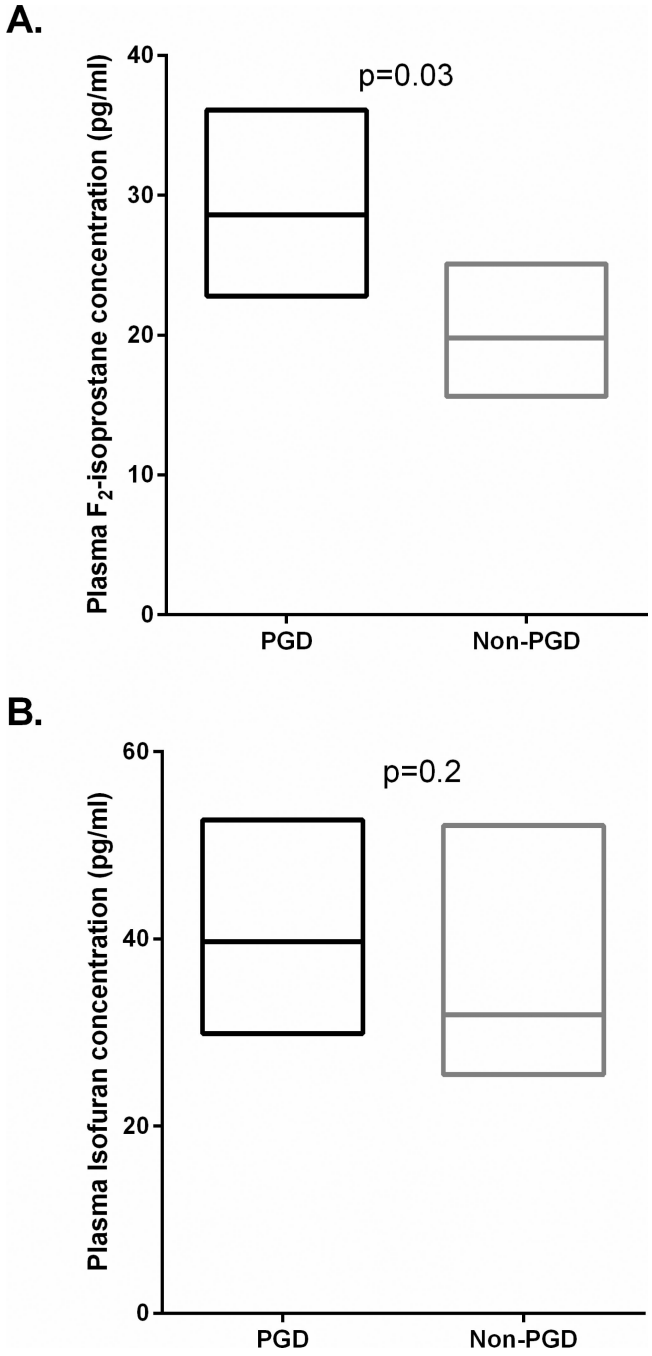

Figure 1.

Box plots of lipid peroxidation product plasma levels. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD.A: Comparison of F_2-isoprostane levels in patients with and without severe PGD; B: Comparison of isofuran levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

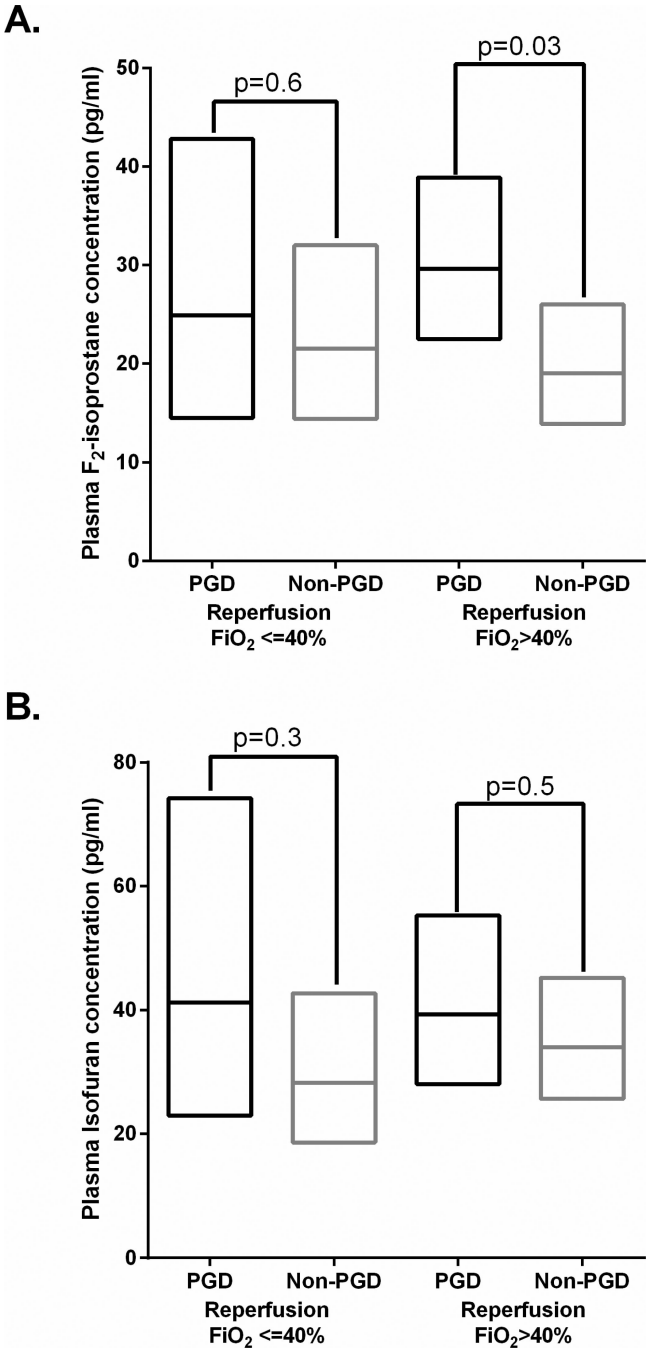

Figure 2.

Box plots of lipid peroxidation product plasma levels with subgroups defined by reperfusion $FiO_2 \leq 40\%$ vs. $FiO_2 > 40\%$. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD. A: Comparison of F_2-isoprostane levels in patients with and without severe PGD; B: Comparison of isofuran levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

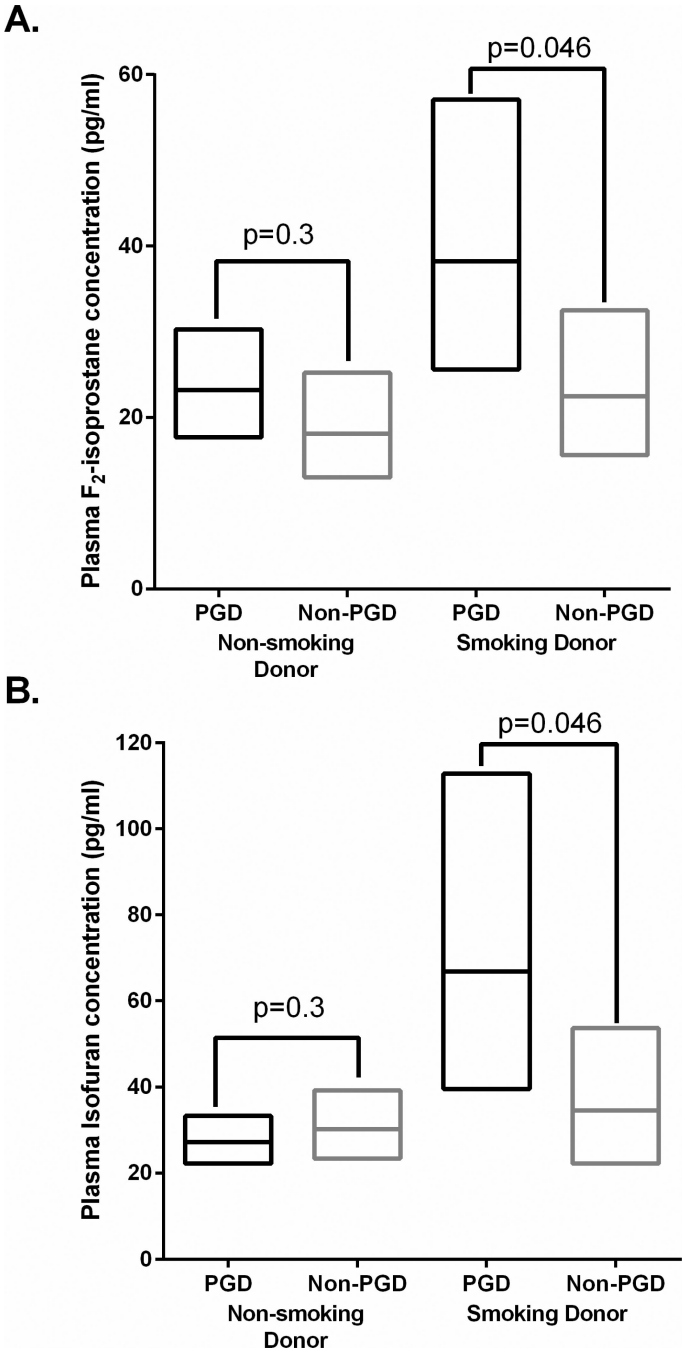

Figure 3.

Box plots of lipid peroxidation product plasma levels with subgroups defined by donor smoking history. Box represents the non-transformed plasma levels corresponding to the mean and 95% confidence intervals of the transformed values. Black boxes are patients with severe PGD and grey boxes are patients without PGD. A: Comparison of F_2-isoprostane levels in patients with and without severe PGD; B: Comparison of isofuran levels in patients with and without severe PGD. P-value represents result of t-test of log-transformed values.

Table 1

Subject Demographics. PGD is defined as grade 3 PGD at day 2 or 3.

Covariate	PGD (n=31)	Non-PGD (n=41)	p-value
Donor Variables			
Male Gender, n (%)	19 (61)	25 (61)	0.9
Age, mean	35.2	35.1	0.9
Race, n (%)			0.4
Caucasian	16 (52)	29 (71)	
African American	9 (29)	6 (15)	
Other	6 (19)	6 (15)	
Any smoking, yes	13 (42)	17 (41)	0.1
Recipient Variables			
Male Gender, n (%)	16 (52)	22 (54)	0.9
Age, mean	53.9	53.9	0.9
BMI, mean	27.4	24.0	0.0008
Pulmonary Diagnosis, n (%)			0.3
Chronic obstructive pulmonary disease	9 (29)	19 (39)	
Idiopathic pulmonary fibrosis	13 (42)	14 (34)	
Cystic Fibrosis	1 (3)	6 (15)	
Other	8 (26)	5 (12)	
mPAP	50.5	44.2	0.2
Race, n (%)			0.2
Caucasian	22 (71)	35 (85)	
African American	8 (26)	4 (10)	
Other	1 (3)	2 (5)	
Operative Variables			
Ischemic time, min	324	332	0.7
Transplant type, single, n (%)	10 (32)	13 (32	0.9
PRBC volume, ml	1282	1015	0.5
Reperfusion FiO_2, %	73	64	0.2
Reperfusion FiO_2 category, n (%)			0.2
21-40%	6 (19)	14 (34)	
>40%	25 (81)	27 (66)	
Cardiopulmonary bypass use, yes, n (%)	18 (58)	17 (41)	0.2

BMI: Body mass index

mPAP: mean pulmonary artery pressure

FiO_2: Fraction of inspired oxygen

Percentages may not exactly equal 100% because of rounding.